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Maternal Viral Load, CD4 Cell Count and Vertical Transmission of HIV-1

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HIV load and CD4 cell numbers were measured among 95 HIV infected women during pregnancy in order to determine their value as prognostic markers for transmission of virus from mother to infant. Among the 94 live births, 13 children were infected with HIV, 69 were uninfected and 12 were of unknown infection status. HIV RNA levels, as measured by nucleic acid sequence based amplification, were significantly higher ($P < 0.001$) in women who transmitted virus than among those who did not transmit and maternal viral load was a stronger predictor of transmission than CD4 cell number. The predicted rate of transmission relative to maternal HIV RNA was 2% at 1,000 copies, 11% at 10,000 copies and 40% at 100,000 copies/ml. Little variation in viral load occurred during pregnancy and there was an association between viral load and prematurity, the mean gestation at delivery decreasing by 1.3 weeks for every 10-fold increase in maternal HIV RNA ($P = 0.007$). This study demonstrates that a high level of maternal HIV RNA is a risk factor for transmission of virus to the infant and maternal viral load is of more value as a prognostic marker for transmission risk than CD4 cell number. High viral load is also associated with premature delivery. Maternal viral load is therefore a useful marker on which to base management decisions during pregnancy. ***J. Med. Virol. 54:113–117, 1998.***

KEY WORDS: HIV load; vertical transmission; CD4 cells

INTRODUCTION

Transmission rates of HIV from mother to infant range from 14 to 40% and advanced maternal clinical and immunological disease, reflecting increased viral load, has been associated with an increased risk of transmission [European Collaborative Study, 1996; Mandelbrot et al., 1996]. Treatment with zidovudine during pregnancy and delivery, and to the infant for 6 weeks, reduces transmission significantly providing indirect evidence of the importance of maternal viral load [Connor et al., 1994; Sperling et al., 1996]. It is only more recently, however, that HIV RNA has been measured during pregnancy, and shown to be associated with vertical transmission risk [Borkowsky et al., 1994; Fang et al., 1995; Zollner et al., 1996; Coll et al., 1997; Thea et al., 1997]. It has also been suggested that there may be a threshold level of maternal RNA below which transmission is unlikely [Fang et al., 1995]. As HIV load appears to be a more accurate predictor of HIV disease progression than CD4 cell count [O'Brien et al., 1996; Ruiz et al., 1996; Saag et al., 1996; Volberding et al., 1996; Henrard et al., 1997], the same may be true for the association with vertical transmission.

In the UK, the offer of named ante-natal HIV testing is actively being encouraged [Department of Health, 1994] and in some centres has resulted in a significant

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TABLE I. Details of 95 Women in Whom HIV Viral Load Was Measured

Details	N
Clinical status	
Symptomatic (CDC stage IV)	9 (15%)
Asymptomatic (CDC stage 11)	86 (90.5%)
Zidovudine treatment during pregnancy	
Yes	12 (12.6%)
No	83 (87.3%)
Pregnancy outcome	
Live birth	94 (98.9%)
Still birth	1 (1.0%)
Mode of delivery	
Vaginal	75 (78.9%)
Elective caesarian section	15 (15.7%)
Emergency caesarian section	5 (5.2%)
Breast feeding	
No	94 (98.9%)
Yes	1 (1%)
HIV infection status of child	
Infected	13 (13.6%)
Uninfected	69 (72.6%)
Unknown	13 (13.6%)

increase in the uptake of testing [Dennison et al., 1996]. As more infected pregnant women are identified there is likely to be an increase in the demand for monitoring viral load during pregnancy. However, further data are required on which to base management decisions, including the significance of maternal viral load as a prognostic marker for vertical transmission. Currently, the number of studies providing such information is limited and have generally involved a relatively small number of women.

We have quantified HIV RNA in plasma samples from 95 HIV infected pregnant women and assessed viral load relative to maternal characteristics including clinical status, CD4 cell numbers and the infection status of the infant. In addition, we also evaluated the variation of viral load during pregnancy.

MATERIALS AND METHODS

Study Population and Specimens

Plasma samples were collected from 95 HIV infected pregnant women and stored at −70°C. Informed consent was obtained from all women and their details are given in Table I. At the time samples were collected nine of the women had symptomatic disease (CDC stage IV), 86 were asymptomatic (CDC stage II) and 12 (five of whom had symptomatic disease) were treated with zidovudine. Women were enrolled in two separate prospective studies evaluating mother to infant transmission of HIV [European Collaborative Study, 1996] and recruited from antenatal clinics in Madrid (n = 60), London (n = 17), Dublin (n = 11) and Dundee (n = 7). Ten percent of samples were collected in the second trimester of pregnancy, 30% in the third trimester and 52% at, or within 1 week of, delivery. The remaining 8% were taken between 1 week and 2 months after delivery. Plasma samples were stored in Madrid and transported to London for testing, and samples from the other centres were processed and stored in London.

Quantitation of HIV RNA and Enumeration of CD4 Cells

HIV RNA was quantified retrospectively in stored plasma samples using the NASBA HIV-1 RNA QT assay (Organon Teknika Ltd.), according to the manufacturer's instructions. The lower detection limit of the assay was 400 RNA copies/100 μl and levels of HIV RNA were expressed as a copy number per ml of plasma. Seventy-one women had HIV RNA quantified in one sample collected during pregnancy or within 2 months post-partum, 21 women had two or three samples tested and three women had four or five samples tested during this period. All analyses of RNA copy number were performed on a logarithmic scale. Enumeration of CD4 cells was conducted locally using flow cytometry and expressed as the number of cells per cubic mm. Where serial samples were available the analysis of transmission risk was based on the mean value of CD4 cell number or HIV-RNA copy number.

Diagnosis of Paediatric HIV Infection

This was based on a combination of assays including virus isolation, DNA PCR, p24 antigen detection and HIV class specific antibody responses, in accordance with the CDC definitions [Centers for Disease Control, 1994]. In general, HIV diagnosis was made when two independent tests on a single sample were positive and this was confirmed in a second specimen, as previously described [McClure et al., 1995]. All tests were performed locally [European Collaborative Study, 1996].

RESULTS

All but one of the 95 pregnancies resulted in live births (Table I). Thirteen of the children were infected with HIV and 69 were uninfected. The remaining 13 children were of unknown infection status, one was a stillbirth and 12 were either lost to follow up or the parents refused any laboratory investigations.

Among the 12 women treated with zidovudine, two transmitted virus to their infant, eight did not transmit, one had a stillbirth and one infant was of unknown HIV infection status.

Among the 13 women who transmitted HIV to their infants two were symptomatic and had been treated with zidovudine during pregnancy; there were no pretreatment samples available from these women. The remaining 11 women were asymptomatic and did not receive antiretroviral treatment. Twelve of these women had vaginal deliveries and none breast fed their infant.

HIV-RNA Levels and CD4 Cell Counts

Women who transmitted virus had significantly higher ($P < 0.001$) mean HIV-RNA levels (40,473 copies/ml, SD 0.61) than those who did not transmit (7,859 copies/ml, SD 0.68). Although mean CD4 cell numbers were lower among women who transmitted HIV (297, SD 184) compared to those who did not (417, SD 253) this difference was not significant ($P > 0.1$). Figure 1

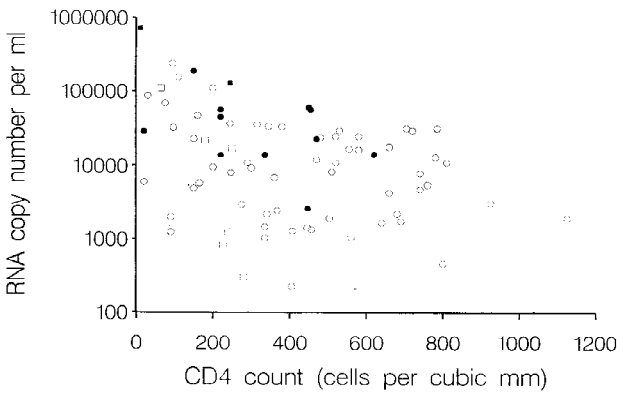

Fig. 1. Maternal HIV-RNA copy number, CD4 cell count and vertical transmission. ○, nontransmitters; ●, transmitters; □, zidovudine treatment.

shows the relationship between maternal viral load, CD4 cell numbers and vertical transmission. HIV RNA copy number was negatively correlated with CD4 cell numbers (correlation coefficient = −0.38, $P < 0.001$), with high viral load being associated with low CD4 cell numbers.

Whether CD4 count was a less effective predictor of transmission than maternal viral load (Fig. 1) was assessed formally by logistic regression analysis (Table II). Because viral load and CD4 cell numbers are measured in different units, comparisons can only be made on a standard deviation scale. For a one standard deviation increase in log RNA copy number (i.e. 0.728 or approximately fivefold) the odds of transmission are increased by a factor of 3.5 (95% CI 1.5–8.2). In contrast, a 1 standard deviation decrease in CD4 cell number (i.e. 261) is associated with only a 1.8 (95% CI 0.9–3.9) increase in the odds of transmission (Table II). HIV RNA copy number is therefore a stronger single predictor of HIV transmission than CD4 cell number. Further analyses were carried out to estimate the effect of each parameter eliminating the effect of the other and this showed that there was little change in the effect of RNA copy number (odds ratio = 3.3), but that CD4 cell number had no predictive value (odds ratio = 1.1) over and above RNA copy number.

Figure 2 shows the predicted rate of vertical transmission by log RNA using the univariate model in Table II, which was 2% at 1,000 RNA copies, 11% at 10,000 copies and 40% at 100,000 copies/ml. The width of the confidence interval reflects the small number of women available, especially at the higher extreme. Vertical transmission occurred in only one of 38 (2.6%) women with less than 10,000 HIV RNA copies/ml (Fig. 1) which is little lower than predicted by the model.

Variation in Viral Load

Variability in viral load during pregnancy was assessed by calculating, for each of 19 zidovudine naive women, the difference in HIV RNA copy number between the first and second samples, the second and third and so on. Among the 31 sequential paired samples the change was less than 0.25 log in 16 (52%), between 0.25 and 0.5 log in nine (29%), and greater than 0.5 log in six (19%). Among the four women whose viral load changed by more than 0.5 log there was no consistent pattern in the direction of the change and CD4 cell numbers remained stable.

Two of the 12 women treated with zidovudine during pregnancy had a pretreatment sample tested and there was no significant change in viral load (<0.3 log).

TABLE II. Predictive Value of Maternal Viral Load and CD4 Cell Number for Vertical Transmission

Parameter	Standard deviation	Increase in odds of transmission per 1 standard deviation change[1]	
		Unadjusted	Adjusted[2]
Log RNA copy no.	0.72	3.5 (1.5–8.2)[3]	3.3 (1.3–8.6)
CD4 cell number	261	1.8 (0.9–3.9)	1.1 (0.5–2.6)

[1]Increase in RNA, decrease in CD4 cell number.
[2]Adjusted for the other parameter.
[3]95% confidence interval.

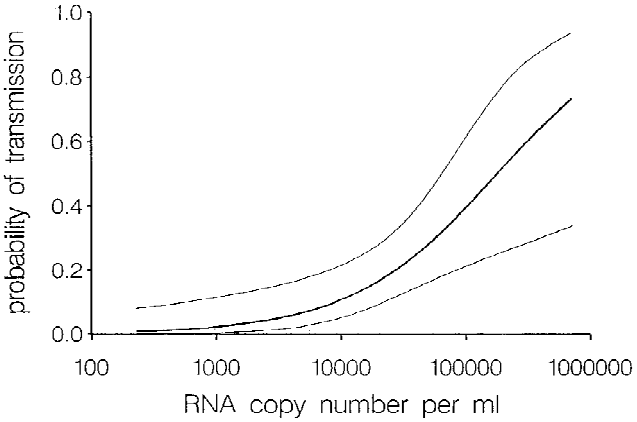

Fig. 2. Influence of maternal viral load on the probability of vertical transmission. Heavy line = estimated risk; light line = 95% confidence interval.

Viral Load and Duration of Pregnancy

We showed previously that women with CD4 counts below 200 cells per mm^3 are significantly more likely to deliver prematurely than women with CD4 cell counts above this level [European Collaborative Study, 1996], and found here a similar association with maternal viral load and premature delivery (Fig. 3). Excluding women who underwent elective caesarian section, mean length of pregnancy decreased by 1.3 weeks for every 10-fold increase in RNA copy number (SE 0.5; $P = 0.007$).

DISCUSSION

We have demonstrated that maternal viral load is a powerful predictor of vertical transmission and more informative than maternal CD4 cell numbers. This contrasts with recent findings [Cao et al., 1997] demonstrating more limited use of maternal viral load in

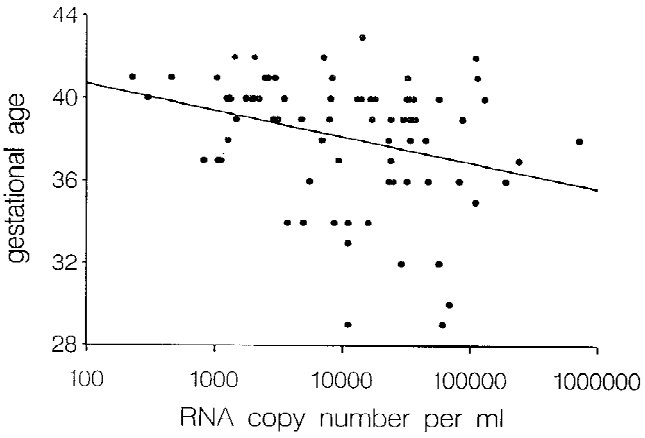

Fig. 3. Maternal viral load and gestational age at delivery.

predicting transmission. Our findings extend the information from natural history studies which demonstrate that viral load is more effective in predicting progression of HIV disease than CD4 cell count. However, recent data from the ACTG 076 zidovudine intervention trial showed that the reduction in vertical transmission was only partly explained by the decrease in maternal plasma HIV RNA [Sperling et al., 1996], which highlights the uncertainty regarding mechanisms of mother-to-child transmission [Scarlatti, 1996].

Intra-assay variation of HIV RNA in plasma is between 0.1 and 0.2 log and the natural biological variation of HIV RNA in plasma is in the order of 0.3 log [Saag et al., 1996]. In practice, therefore, a 0.5 log (threefold) change in viral load is generally considered to indicate a significant change in the level of HIV replication [Saag et al., 1996]. In our study, viral load was relatively stable during pregnancy and the changes that did occur could have been the result of such factors as intercurrent infection. We demonstrated that CD4 cell enumeration failed to contribute additional information on transmission risk. However, to predict progression of disease in individual women, clinicians may prefer to use both viral load and CD4 cell count [Saag et al., 1996].

A number of studies have examined whether there are "thresholds" (high or low) for vertical transmission [Borkowsky et al., 1994; Fang et al., 1995; Dickover et al., 1996; Zollner et al., 1996; Coll et al., 1997; Thea et al., 1997]. We would not like to suggest an absolute level of maternal HIV RNA associated with transmission as we found it occurred over a wide range of maternal RNA levels (~2,000–>100,000 copies/ml) which is consistent with the recent findings of Cao and colleagues [1997]. However, if a level could be identified below which vertical transmission is unlikely to occur, then it could be argued that interventions to reduce transmission [Consensus Workshop Siena, 1995], such as caesarean section or anti-retroviral therapy, would not be justified. We demonstrated a low transmission risk for women with viral loads below 10,000 RNA copies/ml, and the benefits of zidovudine therapy in reducing vertical transmission risk may not outweigh the disadvantages of short term monotherapy in these women [Newell and Gibb, 1995].

The ECS has shown previously that severe premature delivery is associated with low maternal CD4 cell numbers [European Collaborative Study, 1996] and we now confirm an association between premature delivery and increasing maternal viral load. It is likely that both a high viral load and low CD4 cell numbers are associated with the risk of other infections which could induce prematurity. This extends the finding that HIV infected women have an increased risk of adverse pregnancy outcome compared with uninfected controls [Temmerman et al., 1994; Langston et al., 1995]. These findings could have potential implications for the timing of intervention strategies.

The majority of women participating in this study did not receive zidovudine during pregnancy and we were therefore unable to confirm the recent finding [Sperling et al., 1996] that, despite significantly reducing transmission of HIV from mother to baby, zidovudine treatment had only a minimal effect in decreasing the level of maternal HIV RNA. The majority of transmissions from mother to infant occur at or during delivery, consequently viral load in the genital tract may be an important determinant in whether or not transmission occurs. In preliminary studies [O'Shea et al., 1997], in nonpregnant women, we have demonstrated similar levels of HIV RNA in plasma and cervical vaginal secretions. However, further studies are required on the relationship between viral load in the plasma and genital tract of pregnant women which should also include evaluation of the effect of anti-retroviral treatment and development of drug resistance.

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